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Chemical composition of the leaf essential oil of *Lindera benzoin* growing in North Alabama

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Abstract
The leaf essential oil of *Lindera benzoin* (L.) Blume (Lauraceae), growing in Huntsville, Alabama, was isolated by hydrodistillation. This plant, known commonly as spicebush, was used in native American traditional medicine, and is a host plant of the spicebush swallowtail butterfly, *Papilio troilus*. The chemical composition of the leaf oil of *L. benzoin* was determined by GC–MS. The most abundant essential oil components were 6-methyl-5-hepten-2-one (42.9%), β-caryophyllene (7.7%), bicyclogermacrene (5.1%), δ-cadinene (4.9%), and (*E*)-nerolidol (4.8%).

Keywords: Lauraceae, host plant, 6-methyl-5-hepten-2-one, sesquiterpenes

1. Introduction

Lindera benzoin (L.) Blume var. *pubescens* (Palmer & Steyermark) Rehd. "northern spicebush", is a shrub, up to 5 m tall, dioecious. Twigs are olive-green to brown with numerous light lenticels; leaves are alternate, simple, entire, glabrous or pubescent, elliptic to obovate, 7-13 × 2-6 cm, emit a spicy odor when crushed; the bark is brown to gray-brown with light colored lenticels; flowers, in axillary clusters, are small, pale green to yellow; fruit is a bright red oblong drupe, about 10 mm long [1, 2]. *L. benzoin* ranges in moist forests of eastern North America from southern Ontario to Florida. The plant has been used in Cherokee traditional medicine as a tea for treating coughs and colds [3] and is a host plant for the spicebush swallowtail butterfly, *Papilio troilus* L [4]. The essential oil compositions from leaves, twigs, and fruits from cultivated shrubs from Delaware have been reported [5]. This report presents the leaf essential oil composition from an individual growing in Huntsville, Alabama.

2. Materials and Methods

2.1 Plant Material

Leaves of *L. benzoin* were collected from a male individual shrub growing in Huntsville, Alabama (34º 38′ 46″ N, 86º 33′ 27″ W, 187 m elevation) on May 24, 2006. The plant was identified by W.N. Setzer, and a voucher specimen (LIBE-2006-WNS) has been deposited in the University of Alabama in Huntsville Herbarium. The fresh leaves were chopped and hydrodistilled for 4 h using a Likens-Nickerson hydrodistillation apparatus with continuous extraction with $CHCl_3$ (50 mL). The chloroform was evaporated to give the leaf essential oil: 48.33 g fresh leaves gave 74.8 mg (0.155% yield) essential oil.

2.2 Gas Chromatographic – Mass Spectral Analysis

The leaf essential oil of *L. benzoin* was subjected to gas chromatographic-mass spectral analysis using an Agilent 6890 GC with Agilent 5973 mass selective detector, fused silica capillary column (HP-5ms, 30 m × 0.25 mm), helium carrier gas, 1 mL/min flow rate; injection temperature 200 ºC, oven temperature program: 40 ºC initial temperature, hold for 10 min; increased at 3 ºC/min to 200 ºC; increased 2º/min to 220 ºC, and interface temp 280 ºC; EIMS, electron energy, 70 eV. The sample was dissolved in $CHCl_3$ to give a 1% w/v solution; 1-μL injection using a splitless injection technique was used. Identification of oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [6] and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chemstation data system (G1701CA, version C.00.01.08)].

3. Results and Discussion

The chemical composition of *Lindera benzoin* leaf essential oil is presented in Table 1. A total of 37 compounds were identified in the leaf oil accounting for 94.6% of the total composition. *L. benzoin* leaf oil as revealed in this study was dominated by 6-methyl-5-hepten-2-one (42.9%), β-caryophyllene (7.7%), bicyclogermacrene (5.1%), δ-cadinene (4.9%), (*E*)-nerolidol (4.6%), (*E*)-β-farnesene (3.5%), and an unidentified sesquiterpene alcohol (3.8%). The chemical composition is qualitatively similar to the leaf oils of *L. benzoin* var. *benzoin* from Delaware and from Oregon [5], but with notable quantitative differences. 6-Methyl-5-hepten-2-one was particularly abundant in the sample from Delaware (34.8%), as was β-caryophyllene (48.4%) and (*E*)-nerolidol (12.1%). The leaf oil from Oregon, however, had a much lower concentration of 6-methyl-5-hepten-2-one (1.9%). The different compositions can be attributed to very different geographical locations and climatic conditions. *L. benzoin* is a dioecious shrub. The samples from Delaware were female (fruits were collected) [5], but the gender of the shrub from Oregon was not reported [5]. The shrub from Alabama, this work, is male, and gender differences may also contribute to the qualitative differences in essential oil composition.

Table 1: Chemical composition of *Lindera benzoin* leaf essential oil.

RI[a]	Compound	%		RI	Compound	%
860	*cis*-3-Hexenol	0.90		1504	*cis*-α-Bisabolene	0.20
979	Sabinene	0.47		1511	β-Bisabolene	2.84
995	6-Methyl-5-hepten-2-one	42.94		1514	γ-Cadinene	1.64
1011	α-Phellandrene	1.81		1525	δ-Cadinene	4.92
1028	*p*-Cymene	0.25		1537	α-Cadinene	0.15
1034	1,8-Cineole	1.18		1543	*trans*-α-Bisabolene	0.48
1106	6-Methyl-3,5-heptadien-2-one	0.29		1567	(*E*)-Nerolidol	4.57
1155	Citronellal	0.53		1576	Unidentified[b]	3.75
1231	Citronellol	0.48		1581	Caryophyllene oxide	0.98
1336	δ-Elemene	0.28		1641	τ-Cadinol	1.17
1356	Citronellyl acetate	0.22		1645	α-Muurolol	0.21
1384	β-Bourbonene	0.16		1654	α-Cadinol	1.22
1392	β-Elemene	0.98		1684	α-Bisabolol	1.32
1409	α-Gurjunene	0.17		1722	(*E*,*E*)-Farnesol	1.87
1420	β-Caryophyllene	7.74		1784	β-Bisabolenol	2.33
1436	α-*trans*-Bergamotene	1.14		1816	(*Z*,*E*)-Farnesyl acetate	0.85
1452	α-Humulene	0.70			Monoterpene hydrocarbons	2.53
1460	(*E*)-β-Farnesene	3.48			Oxygenated monoterpenoids	2.40
1476	γ-Muurolene	0.25			Sesquiterpene hydrocarbons	30.99
1480	Germacrene D	0.13			Oxygenated sesquiterpenoids	18.27
1497	Bicyclogermacrene	5.12			Others	44.13
1500	α-Muurolene	0.62			Total Identified	94.57

[a] RI = Retention Index, determined with reference to a homologous series of normal alkanes on an HP-5ms column.
[b] Unidentified sesquiterpene alcohol, MS (EI): 222(0.3%), 204(26%), 189(7%), 161(100%), 147(6%), 133(18%), 119(39%), 105(61%), 91(38%), 81(31%), 67(9%), 55(12%).

It is not currently known if volatile leaf compounds serve to attract *P. troilus* adults to their host plants. It is known that tarsal contact of Lepidopterans with the host plant surface is necessary to initiate oviposition [7]; contact chemoreceptors on the tarsi are stimulated by particular phytochemicals and elicit oviposition [8-10], but host plant volatiles as well as visual cues likely contribute to host plant selection in *Papilio* spp.[11, 12]. Host plants of *P. troilus* are restricted to the Lauraceae and include *Sassafras albidum*, *Lindera melissifolia*, and *Persea borbonia* in addition to *L. benzoin* [13]. *S. albidum* leaf oils are dominated by geranial (11-27%) and neral (10-18%), and β-caryophyllene (5-13%), with lesser amounts of (*E*)-nerolidol and δ-cadinene, but no detectable quantities of 6-methyl-5-hepten-2-one or bicyclogermacrene [14]. *P. borbonia* leaf oil, on the other hand, is dominated by camphor (35%) and 1,8-cineole (18%) [15]. There have apparently been no reports on leaf essential oil composition of *L. melissifolia*, but the volatiles from the fruits have been reported [16].

4. Conclusions

The leaf essential oil composition of *Lindera benzoin* var. *pubescens* from north Alabama has been determined. The oil was dominated by 6-methyl-5-hepten-2-one (44%), with lesser quantities of β-caryophyllene (8%), bicyclogermacrene (5%), δ-cadinol (5%), and (*E*)-nerolidol (5%). This work complements a previous study of *L. benzoin* var. *benzoin* from Delaware and Oregon [5].

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